A Kallikrein-Like Enzyme in the Aorta of Normotensive and Hypertensive Rats

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Abstract We evaluated whether vascular kallikrein is altered in rats with genetic or experimental hypertension. Group 1 was infused intraperitoneally with angiotensin II (Ang II) or vehicle for 4 weeks; group 2 was injected subcutaneously with deoxycorticosterone (75 μmol/kg once a week) or vehicle for 4 weeks; group 3 consisted of uninephrectomized rats on high sodium intake given deoxycorticosterone or vehicle; and group 4 consisted of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Active and total kallikrein activity was measured in abdominal aortic homogenates using an amidolytic assay. Ang II increased systolic blood pressure at a dose of 400 nmol/kg per day (146±5 versus 123±3 mm Hg in controls, P<0.01) but not at 80 nmol/kg per day. Deoxycorticosterone did not increase blood pressure except in uninephrectomized rats on high salt diet (173±6 versus 135±4 mm Hg in controls, P<0.01). Blood pressure averaged 194±2 mm Hg in SHR and 123±3 mm Hg in WKY rats. Vascular kallikrein was similar in rats given Ang II or vehicle. In deoxycorticosterone-treated rats total kallikrein was higher than in controls (9.2±0.8 versus 3.5±0.1 pkat/mg protein, P<0.05), whereas active kallikrein did not differ (0.09±0.04 versus 0.09±0.03 pkat/mg protein, P=NS). A similar pattern was observed in uninephrectomized deoxycorticosterone-treated rats (active, 0.09±0.03 versus 0.10±0.04, P=NS; total, 7.4±0.7 versus 4.1±0.2 pkat/mg protein, P<0.05). Kallikrein activity was higher in SHR compared with WKY rats (active, 0.34±0.04 versus 0.10±0.03; total, 9.5±1.2 versus 4.6±0.3 pkat/mg protein, P<0.05). In conclusion, a kallikrein-like enzyme is present in rat aorta. The activity of this enzyme is elevated in rats with genetic hypertension, and it may be regulated by mineralocorticoids.

Key Words: • blood pressure • bradykinin • angiotensins • mineralocorticoids

Methods

Male Wistar rats (Morini), SHR, and Wistar-Kyoto (WKY) rats (Charles River) weighing between 220 and 240 g were housed at a constant room temperature with a 12-hour light/dark cycle and had free access to rat chow. The experimental protocol was approved by the local Animal Care and Use Committee. All procedures complied with the standards for the care and use of animals as stated in Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, Md). Surgery was performed with rats under ether anesthesia; disappearance of the corneal reflex was used to adjust the depth of anesthesia.

Kallikrein activity was measured in homogenates of abdominal aorta of rats that underwent the following experimental protocols.

Experiment 1: Effect of Ang II on Vascular Kallikrein Activity

Ang II (Sigma Chemical Co) at a dose of 80 or 400 nmol/kg body wt per day (n=10 each dose) or saline solution (vehicle, n=10) was infused in Wistar rats for 4 weeks with the use of 2ML4 Alzet osmotic pumps (Alza Corp), which were implanted intraperitoneally.

Experiment 2: Effect of Deoxycorticosterone on Vascular Kallikrein Activity

DOC enantate (Shering) at a dose of 75 μmol in 100 μL saline per kilogram body wt (n=12) or saline (n=12) was injected subcutaneously in Wistar rats once a week for 4 weeks. The same protocol was performed in two additional groups (n=12 each) that underwent unilateral nephrectomy (before the administration of DOC or saline was started) and were given saline solution (0.15 mol/L NaCl) to drink.
Experiment 3: Vascular Kallikrein Activity in SHR and WKY Rats

Kallikrein activity was determined in abdominal aortic homogenates of 10-week-old SHR (n=12) and compared with that of age-matched WKY rats (n=12).

Blood Pressure Measurement

At the end of the experimental period systolic blood pressure (SBP) was measured by tail-cuff plethysmography with a W+W Recorder 8002 (Ugo Basile, Biological Research Apparatus) in conscious rats prewarmed at 37°C.

Analytic Procedures

With rats under ether anesthesia the abdominal aorta was removed, rinsed several times with 0.15 mol/L NaCl, and lightly shaken in an automatic shaker. The saline solution was changed five times, and then vascular pieces were cleaned of adipose and connective tissues, blotted, weighed, minced, and homogenized with a mechanical homogenizer (MSE Braun) and subsequently with an ultrasonic disintegrator (MSE Ltd). The homogenates were centrifuged twice at 2500g. All above procedures were homogenized at 4°C. Supernatants were then stored at -40°C until assay. The protein concentration in the supernatant of vascular homogenates was measured with the method of Lowry et al.6

Glandular kallikrein activity of vascular homogenates was measured by an end point amidolytic method7 at 37°C in 0.1 mol/L Tris-HCl buffer (pH 8.2) containing soybean trypsin inhibitor (15 μmol/L, Sigma). The latter substance was included in the incubation buffer to inhibit plasma kallikrein and trypsinlike enzymes that could be present in the homogenates. The reaction was started by addition of substrate (r>Val-Leu-Arg-p-nitroanilide, S2266, Kabi Diagnostica) at a final concentration of 1.5 mmol/L. Each experimental sample had three controls: (1) homogenate incubated without substrate (blank), (2) homogenate incubated with substrate in the presence of aprotinin or polyclonal antibodies against rat glandular kallikrein (13 nmol/L). Kallikrein antibodies were kindly provided by Dr Julie Chao (University of South Carolina). The color developed during the incubation was measured at 405 nm every hour for 8 hours (Perkin-Elmer). Then the reaction was stopped by addition of 100 μL of 50% (vol/vol) acetic acid. The absorbance of blanks was subtracted from that of corresponding experimental samples, and kallikrein activity was expressed in picokatals (1 pkat represents the enzyme activity able to cleave 1 pmol p-nitroaniline per second from substrate).

Inactive kallikrein was activated according to a previously published method8 by incubating 500 μL of the homogenates with 50 μg Sepharose-bound trypsin; the latter was removed after 8 hours by centrifugation (250g for 20 minutes). Kallikrein activity was then measured in trypsin-treated homogenates with the amidolytic assay. Because enzymatic activity was much higher after trypsin treatment, vascular homogenates had to be appropriately diluted to ensure that less than 10% of the substrate was hydrolyzed at the end of the incubation.

Data are expressed as mean±SEM. ANOVA was used to compare kallikrein levels in homogenates of experimental and control groups. Mathematical calculations and statistical analysis were performed with a STATVIEW II package (Brain Power) on an Apple Macintosh IIICX computer.

Results

SBP was similar in rats given 80 nmol Ang II or vehicle (123±3 versus 123±4 mm Hg, P=NS), whereas it was significantly higher in rats given 400 nmol (146±6 mm Hg, P<.01).

As shown in the Figure, SBP of rats given DOC alone did not differ from that of controls (133±3 versus 127±4 mm Hg in controls, P=NS). By contrast, DOC combined with high salt intake increased SBP in uninephrectomized rats (173±6 versus 135±4 mm Hg in controls, P<.01). SBP averaged 194±2 mm Hg in SHR and 123±3 mm Hg in WKY rats (P<.01).

The rate of p-nitroaniline release during incubation of vascular homogenates with S2266 was linear through time. The enzymatic activity was completely blocked when homogenates were incubated with S2266 in the presence of aprotinin or polyclonal antibodies against rat glandular kallikrein.

The kallikrein-like enzyme was present mainly in the inactive form. Similar levels were found in the homogenates of rats given 80 or 400 nmol Ang II or vehicle (active, 0.09±0.03, 0.09±0.03, and 0.10±0.04 pkat/mg protein; total, 3.02±0.22, 5.43±1.74, and 4.66±1.43 pkat/mg protein, respectively).

As shown in the Figure, total kallikrein was higher in DOC-treated rats compared with controls (9.20±0.80 versus 3.50±0.10 pkat/mg protein, P<.05). However, active kallikrein did not differ between groups (0.09±0.04 versus 0.09±0.03 pkat/mg protein, P=NS).

A similar pattern was observed in uninephrectomized DOC-treated rats (active, 0.09±0.03 versus 0.10±0.04 pkat/mg protein in controls, P=NS; total, 7.44±0.71 versus 4.10±0.24 pkat/mg protein in controls, P<.05).

Kallikrein activity was higher in SHR compared with WKY rats (active, 0.34±0.04 versus 0.10±0.03 pkat/mg protein; total, 9.48±1.20 versus 4.60±0.28 pkat/mg protein, P<.05).
Discussion

The present study confirms the presence of an enzyme resembling glandular kallikrein in the active and trypsin-activatable form in rat aorta. In addition, our data indicate that the activity of this enzyme is increased in the abdominal aorta of rats given chronic infusion of DOC and in SHR, whereas it is not altered in angiotensin-treated rats.

Previous studies have shown that the amidolytic assay with S2266 is an adequate method for measurement of vascular kallikrein. This assumption is inferred from the linearity of the generation rate with time as well as from the correlation between amidolytic and kininogenase activities. Resistance to soybean trypsin inhibitor discounts the possibility that plasma kallikrein or other trypsinlike enzymes are responsible for the ability of vascular homogenates to cleave the synthetic substrate S2266. In addition, inhibition of the enzymatic activity by aprotonin and polyclonal antibodies against rat glandular kallikrein favors the possibility that the amidolytic activity measured in vascular homogenates is attributable to an enzyme belonging to the glandular kallikrein family. It seems unlikely that glandular kallikrein derived from plasma (trapped in the tissue during surgical dissection) is responsible for the enzymatic activity of vascular homogenates, because vessels were extensively washed before the assay. Persistence of kininogenase activity after repeated washing was previously reported by Nolly et al and our group. Although the possibility that circulating glandular kallikrein enters the vascular wall cannot be completely excluded, the presence of messenger RNA for glandular kallikrein in the vascular wall and in an established line of rat vascular smooth muscle cells supports the hypothesis that glandular kallikrein is synthesized within the wall vasculature. However, the layer responsible for the synthesis of kallikrein has not been identified. Oza et al reported that rat vascular smooth muscle cells in culture release kinins, and vascular kininogenase activity is still present in rat vascular homogenates after endothelium removal by collagenase. However, the finding that endothelial cells in culture are able to release bradykinin suggests that kallikrein may be located in both endothelial and smooth muscle cells.

In preliminary experiments kallikrein activity was measured in pooled vascular homogenates by the kininogenase method (P.M., unpublished observations). Kininogenase activity found in the aorta of normotensive rats (active, 5.1±0.4 and inactive, 230±22 pg bradykinin/mg protein per minute, n=7) was lower compared with the levels reported by Nolly et al but similar to that observed by our group in homogenates of human aorta.

Vascular kallikrein activity is decreased in the arteries of one-kidney, one clip hypertensive rats, whereas the present study indicates that it is not altered by chronic administration of Ang II. Thus, reduced vascular kallikrein activity might be typical of volume-dependent hypertension. However, in another volume-dependent experimental model resembling human hypermineralocorticidism, we found that trypsin-activatable vascular kallikrein is increased. Activation of the renal kallikrein-kinin system by mineralocorticoids could represent an important compensatory response to counteract their vasopressor and salt-retaining effects. Indeed, chronic blockade of bradykinin-receptors reportedly causes hypertension in rats with an excess of mineralocorticoids. This effect may be attributable to inhibition of kinins generated within the kidney as well as within the vascular leading to predominance of vasopressor systems.

The finding that kallikrein activity is increased in the aorta of SHR at 10 weeks of age was unexpected, because in patients with essential hypertension vascular kallikrein levels are similar to those of normotensive subjects. Interestingly, kininogenase activity is reportedly depressed in thoracic aorta homogenates of SHR at 15 weeks of age. Thus, reduction in vascular kallikrein content with aging might be secondary to the progression of structural changes occurring in the arterial wall in relation to the duration of hypertension. Alternatively, discrepancies might be explained by differences in the expression of the enzyme between species as well as among SHR substrains.

Although it is tempting to speculate that decreased kallikrein activity may contribute to increased blood pressure in some experimental models and that enhanced activity may prevent or delay the development of hypertension in others, the possible relation between kallikrein activity in the aorta and blood pressure levels should be examined with caution. An overall limitation of our study is that kallikrein activity measured in the aorta might not reflect the activity of the system in resistance vessels. In addition, the release of active kallikrein in different hypertensive conditions was not addressed. Thus, whether important differences in active kallikrein release can be linked to hypertension remains unknown. Finally, as vascular endothelial damage has been hypothesized to convert bradykinin from a vasodilator to a vasoconstrictor, the hemodynamic consequences of alterations in vascular kallikrein activity could depend on the integrity of vascular endothelium.

Recently, a role has been proposed for kinins in mediating the protective effect of converting enzyme inhibitors on neointima formation after balloon injury to the rat carotid. It was of interest to know whether alterations in vascular kallikrein activity can affect the mechanisms involved in neointima formation as well as the efficacy of therapeutic interventions.

In conclusion, a kallikrein-like enzyme is present in rat aorta. Its activity appears to be altered in rats with genetic hypertension, and it may be regulated by mineralocorticoids.

Acknowledgments

This work was supported in part by a grant from the Minister of Universities and Scientific Research and a research grant from the National Research Council (CNR) targeted project "Prevention and Control of Disease Factors" No. 91.001173.41. 

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Hypertension. 1994;23:899-902
doi: 10.1161/01.HYP.23.6.899

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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